Fusarium oxysporum f. sp. *melonis:* A Case Study of Diversity Within a Forma Specialis

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Mycologists and plant pathologists have long been aware that the anamorphic species *Fusarium oxysporum* Schlechtend.:Fr. contained wide phenotypic, and thus presumably genotypic, diversity (7,22). The economic importance of the species has required workers to carefully examine the morphologically indistinguishable isolates that fall into this taxonomic caldron and to attempt to find natural groupings that are both practicably useful and systematically meaningful. The forma specialis and race concepts (1,22), based on pathogenicity, have remained the only widely used subdivisions of *F. oxysporum.* Controversy and differences of opinion exist, even here, due to phenotypic instability and differing methodologies (see, for example, 1,2,21).

Puhalla's recent discovery and application of vegetative compatibility as a stable genetic marker within asexual fungi (reviewed in 8) has generated renewed interest in the sub-forma specialis systematics of *F. oxysporum.* The use of protein and nucleic acid polymorphisms as traits has added a new dimension in the recognition and classification of genetic variability. A comprehensive review of recent advances in genetic and molecular systematics of *F. oxysporum* is beyond the scope of this paper. Consequently, we will limit our discussion primarily to our own work on *F. o. melonis* W. C. Snyder & H. N. Hans. (13–15), using this information to illustrate how genetic and molecular studies can contribute to a better understanding of the relationships within a forma specialis.

Analysis of *F. o. melonis.* *F. o. melonis* causes Fusarium wilt of muskmelon (*Cucumis melo* L.) and has been divided into four races based on three differential cultivars, two of which carry single dominant resistance genes (*Fom*1 and *Fom*2) (21). The races (0, 1, 2, and 1,2) are named for the resistance genes they overcome; these four races are the maximum that can be described with only two resistance genes. Race 1,2, however, is further subdivided into wilt (1,2w) and yellows (1,2y) strains based on symptomatology.

Fusarium wilt of muskmelon has been reported in North America, India, East Asia, and the Middle East, but the distribution of races is not uniform (summarized in 14,15). Before 1985, only race 2 was found in North America, and races 0 and 1 in Europe and Israel. Between 1985 and 1987, races 0 and 1 were reported in Maryland, race 0 in the Rio Grande Valley in Texas and Mexico, and race 1 in Jalisco, Mexico.

The well-defined races and the geographic distribution of *F. o. melonis* made it suitable for the general study of systematic relationships within a forma specialis. In addition, *F. o. melonis* provided an opportunity to examine two specific questions. What are the relationships among strains of the same race isolated from different geographic areas? What is the origin of the races newly discovered in North America and what are their relationships to established infestations?

As a first step, vegetative compatibility groups (VCGs) were identified by use of nitrate nonutilizing (*nit*) mutants in an attempt to distinguish genetically distinct populations within *F. o. melonis* (13,14). A total of 176 strains were assigned to eight different VCGs (Table 1). Twelve additional strains were vegetatively self-incompatible; no heterokaryon was formed between complementary *nit* mutants of these strains or with the *nit* mutant tester pairs of all eight VCGs. Therefore, these strains could not be assigned to a VCG (Table 1).

The relationship between race and VCG proved to be complex. All four races were found in more than one VCG. Differences in vegetative compatibility phenotype indicated that no race represents a genetically homogeneous group of strains. In contrast, all four races also were present in a single VCG. Adaptation to host resistance may be considered as a possible explanation. However, multiple races of *F. o. melonis,* and perhaps multiple VCGs, were present in France before the introduction of resistant cultivars and any accompanying selection pressures (3).

VCG diversity within and between geographic regions also was found. The collection from France includes four separate VCGs. In the San Joaquin Valley of California, different VCGs of the same race have overlapping distributions and were occasionally isolated from the same field. The races new to North America belong to the predominantly European VCG 0134, suggesting that these strains were introduced from Europe and not derived from existing race 2 populations or indigenous nonpathogens.

F. oxysporum strains within a VCG presumably share the identical vegetative incompatibility genotype, given the analogy with sexually reproducing fungi. However, strains in different VCGs may differ at one or many of the loci affecting vegetative compatibility. Thus, vegetative incompatibility is a strictly qualitative trait that reveals strains to be different but provides no measure of the extent of the difference.

Molecular traits, especially polymorphisms in nuclear and mitochondrial DNA (mtDNA), recently have been used to assess interspecific and intraspecific relationships in fungi, including some plant pathogens (cited in 15,24). Variability in mtDNA is of course associated with nuclear traits by an asexual life cycle (no recombination), but may be evolving at a different rate (24). With mtDNA polymorphisms in *F. o. melonis* we were able to quantify relationships among VCGs, clarify the status of self-incompatible isolates within the forma specialis, and provide further insight into the associations between VCG, race, and geographic distribution (15).

Seventy-eight *F. o. melonis* strains, selected from the vegetative compatibility study to represent the extent of race diversity and geographic distribution, were initially surveyed for restriction fragment length polymorphisms in mtDNA. Using three endonucleases, seven distinct restriction fragment length patterns were visualized. These patterns were correlated to VCG. Six VCGs had a unique pattern whereas two VCGs shared the seventh pattern (Table 1). Eleven of the 12 self-incompatible strains had a restriction fragment pattern identical to one of two VCG patterns (C or D, Table 1). The pattern of the remaining strain was unique, but appeared similar to pattern D, differing by a single band of smaller size.

Although informative, visualized restriction patterns, like vegetative compatibility, reveal only qualitative differences between strains. A more detailed characterization of the actual differences among the mtDNAs was required to estimate relationships between the patterns. Differences among the strains consisted of length (insertions and deletions) and restriction site changes. Where multiple changes could have been the result of a single event, it was considered as only one change. For example, the loss of a restriction site and a length decrease may have been the result of a deletion containing that particular site. This conservative approach yielded a total of 27 changes that were used as characters; each strain was scored for presence or absence of these characters. The resulting matrix of character states listed the least number of character changes that would explain the observations.

These data were analyzed by three methods: parsimony, the relationships among isolates that are based by the fewest character changes (11); compatibility, the relationships based on only those characters without character state reversals (11); and phenetics, the relationships based on overall similarity regardless of specific character changes (23). These different analyses showed essentially the same relationships between mtDNA patterns; the most parsimonious unrooted tree is shown in Figure 1.

The apparently strong correlation between VCG and mtDNA may be due, in part, to the small number of strains available for some VCGs. However, even where 30 strains in a VCG were examined, no polymorphisms were identified. The lack of polymorphism in mtDNA within a VCG is striking, especially where virulence diversity exists (VCG 0134). In addition, the distance between VCGs 0130/0131 and VCG 0134 further supports the hypothesis that the recently discovered races new to North America were introduced and not derived from the indigenous populations. The considerable distance between race 1 strains in VCG 0134 and VCG 0136 also argues for separate lineages, and thus a polyphyletic origin of this race.

Wider considerations. Two interrelated issues must be considered when interpreting results from this type of study, especially in the larger context of the entire species: the hidden diversity within the traits used to judge relationships, and the use of these calculated relationships to infer phylogeny. There are both technical and conceptual questions and, again, *F. o. melonis* will serve to provide examples.

Host specialization historically has been considered the most important trait in *F. oxysporum* and, because of its practical application, has been used as the basis of classification. The multiple races suggest considerable virulence diversity within *F. o. melonis,* but it is not clear that this is the most meaningful basis for classifying sub-forma specialis grouping. The race groupings in *F. o. melonis* conceal known diversity in symptomatology, vegetative compatibility, and mtDNA polymorphism. Moreover, the present method of categorizing races may not reveal the total virulence diversity within a taxon. As mentioned above, races are recognized by interaction with resistant cultivars, so that with two resistant genes, the four known races in *F. o. melonis* represent the maximum that can be described by the present system. If additional host resistance genes were available, more races might be identified, possibly changing our perception that different VCGs are identical in virulence. Finally, it is currently impossible to characterize the genetic basis for the virulence phenotypes in asexual fungi such as *F. oxysporum,* thus it is difficult to establish the weight that should be accorded to this character.

TABLE 1. Vegetative compatibility groups, race, mitochondrial DNA restriction pattern, and distribution of *Fusarium oxysporum* f. sp. *melonis*

VCG[a]	Race	mtDNA pattern[b]	Number of strains[c]	Location
0130	2	A	95 (14)	California
0131	2	A	24 (8)	North America
0132	2	B	1	Japan
0133	1,2w	C	9	France
0134	0	D	11 (9)	France, Italy, Maryland, Texas
	1	D	26 (15)	France, Maryland
	2	D	1	Italy
	1,2y	D	5	France
0135	0	E	2	France, Israel
0136	1	F	1	Mexico
0137	?[d]	G	1	France
013-	0	D	3	France
		D_1	1	France
	1	D	4	France, Israel, Maryland
	1,2y	D	2	France
	1,2w	C	2	France
Total			188 (78)	

[a] Vegetative compatibility group. 013-: An artificial group containing vegetatively self-incompatible strains.
[b] Pattern assigned by visual comparisons of mtDNA using three restriction endonucleases. Subscript indicates a similar pattern (see text).
[c] A number in parentheses represents a subset of strains used to determine mtDNA pattern.
[d] This strain was received as *F. o. melonis*, but was avirulent in these studies.

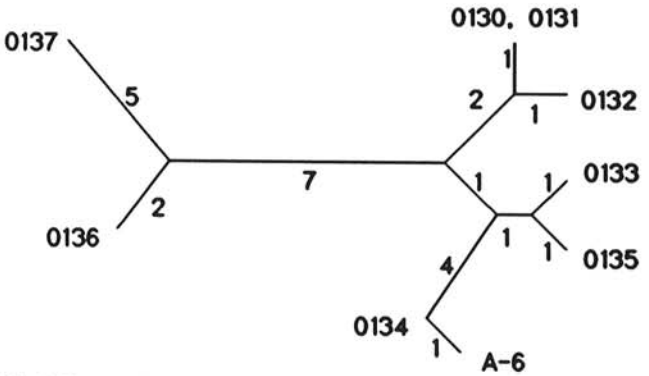

Fig. 1. Unrooted most parsimonious tree of relationships among vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *melonis* based on polymorphisms of mtDNA. A-6 is a vegetatively self-incompatible strain with a unique restriction fragment pattern of mtDNA (see Table 1). The number on each branch represents the number of character changes between nodes.

Traits, other than virulence, that can be used to group strains within *F. o. melonis* also provide only partial measures of the underlying diversity. The correlation between mtDNA and VCG helped confirm the importance of VCG as an indicator of genetically isolated populations within *F. o. melonis* and served as the foundation from which sub-forma specialis relationships were calculated. However, the race diversity within a VCG or mtDNA framework should not be ignored. Studies using isozymes, random DNA fragments, and repetitive DNAs also have shown additional genetic polymorphism within VCGs (10,16,20). Thus, there probably is no one trait, either physiological, genetic, or molecular, that alone will provide an entirely satisfactory basis for classification at any taxonomic level. Although systems for intraspecific taxonomy and terminology have been proposed (4), there is insufficient understanding of the genetic structure in this asexual species to assign strains to even nonhierarchical biotypes, especially when such groupings would overlap. We would suggest, however, that for the present *F. oxysporum* strains should be described by both pathogenicity and VCG, since this is feasible and reveals much more information than either alone.

A considerable amount of new data characterizing the diversity within *F. oxysporum* is providing new perspectives on phylogenetic relationships within the species. Inferring phylogeny from these data, however, is not without problems (11). For example, the major source of variation in fungal mtDNA consists of length differences (insertions and deletions); the evolutionary significance of these differences is unclear. The molecular clock hypothesis, which underlies the phylogenetic analysis of restriction site changes, is not applicable to length variation. Until the basis for the evolution of length changes is understood, statistical analysis and phylogenetic inferences may seem arbitrary. Nevertheless, length changes have proven to be a valuable source of variation for fungal evolutionary studies when a conservative approach is used to assign characters (25).

All phylogenies from molecular data assume that change in a molecule accurately reflects change in the whole organism. This assumption becomes more critical as the portion of the molecule being examined becomes smaller. For instance, an alternative to length variation and restriction site changes is the direct sequencing of a limited portion of the genome. It must be remembered, however, that the evolution of a sequence, not the organism, is truly under study. The assumption that a sequence is representative of the organism ultimately must be verified by broader study of the organism.

The statistical methods used to calculate phylogeny may affect the outcome of the analysis (11,23). For example, the relationships within *F. o. melonis* varied slightly when different phenetic algorithms were used (data shown in 15). The relationships calculated by one algorithm matched both the parsimony and compatibility trees, but this was not sufficient reason to judge one method more correct than the other. Consensus using different methods cannot be used to confirm statistical or biological validity. One algorithm, however, was considered preferable because the method of data collection better fulfilled the statistical assumptions (23). The choice of methodology, therefore, should be based on experimental and biological considerations, rather than whether the outcome fits the expectations.

A variety of models have been proposed to explain the evolution of formae speciales, races, and VCGs in *F. oxysporum* (8,18). Discussion has centered on the timing and relative importance of genetic isolation, as exemplified by the loss of sexual reproduction or development of vegetative incompatibility, and host specialization at different levels. Newer models have generally revised Puhalla's view of VCGs tightly correlated to pathotypes in order to account for the more complex patterns that have emerged. There is no reason to believe that only one model should be correct.

Invoking more than one scenario may, in fact, be the only way to explain the present diversity in *F. o. melonis*. Relationships based on mtDNA polymorphisms suggest that both host specialization and genetic isolation probably occurred repeatedly, but at different levels, in progenitor populations of what is currently recognized as *F. o. melonis*. For example, the distantly related VCGs could be explained by the loss of sexuality, fixing VCG genotype, followed by convergence in host specialization (as a muskmelon pathogen). Host specialization after genetic isolation of a VCG, at a lower taxonomic level, is illustrated by the multiple races within VCG 0134. In contrast, host specialization before genetic isolation is apparent where identical virulence and mtDNA is present in two distinct VCGs (0130 and 0131). This could be explained by the recent derivation of one VCG from the other as a result of a mutation in a gene affecting vegetative compatibility. It is unclear, in all of these cases, whether virulence evolves at a faster rate than other traits, as postulated in other fungi (5,6). The interpretations of phylogenetic relationships in *F. oxysporum* are not purely academic exercises (17). For instance, no race of *F. o. melonis* constitutes a genetically homogenous group. For this reason, we have suggested that the selection of strains used in screening germ plasm for resistance should be carefully considered (13).

The results from our study have made it possible to answer specific questions about this forma specialis. For broader questions, such as those concerning the origin of the forma specialis, answers remain elusive. For example, the unrooted trees of *F. o. melonis* cannot be used to conclude a monophyletic origin for the forma specialis. However, now that relationships within *F. o. melonis* are better understood, this question can be directly addressed by including a true outgroup in future analyses.

Characterization of strains in other formae speciales will reveal more about relationships within these subspecific groups and facilitate comparisons between formae speciales (2,10,16,17, 19,20). In addition, because some of these formae speciales are pathogenic on related hosts (*F. o. melonis, F. o. niveum,* and *F. o. cucumerinum* on the Cucurbitaceae), we may gain insight into the coevolution of host and pathogen. However, our view of *F. oxysporum* ultimately must be expanded to encompass the nonpathogenic strains whose potential importance has long been recognized, but whose diversity has only begun to be characterized (9,12). This should provide a more complete understanding of this economically important species and contribute to more effective management of the diseases for which it is responsible.

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